PCP
PLANT & CELL PHYSIOLOGY

Local Root Apex Hypoxia Induces NO-Mediated Hypoxic Acclimation of the Entire Root

Sergio Mugnai[1], Elisa Azzarello[1], Frantisek Baluška[2] and Stefano Mancuso[1,*]

[1]LINV, Department of Plant, Soil and Environmental Science, University of Florence, Viale delle Idee 30, 50019 Sesto Fiorentino (FI), Italy
[2]Department of Plant Cell Biology, Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany
*Corresponding author: E-mail, stefano.mancuso@unifi.it; Fax, +39-0554574017.

Roots are very sensitive to hypoxia and adapt effectively to a reduced availability of oxygen in the soil. However, the site of the root where oxygen availability is sensed and how roots acclimate to hypoxia remain unclear. In this study, we found that the root apex transition zone plays central roles in both sensing and adapting to root hypoxia. The exposure of cells of the root apex to hypoxia is sufficient to achieve hypoxic acclimation of the entire root; particularly relevant in this respect is that, of the entire root apex, the transition zone cells show the highest demand for oxygen and also emit the largest amount of nitric oxide (NO). Local root apex-specific oxygen deprivation dramatically inhibits the oxygen influx peak in the transition zone and simultaneously stimulates a local increase in NO emission. The hypoxia-induced efflux of NO is strictly associated with the transition zone and is essential for hypoxic acclimation of the entire root.

Introduction

The perception of the local oxygen concentration is a key physiological process that represents the first step of the plant cellular response to low oxygen availability in the soil. As the root apex is the first part of the plant to come into contact with new and previously unexplored regions of the soil, the tip, acting as a dynamic sensory organ, should represent the most probable and obvious site for the sensing process (Aiken and Smucker 1996, Dat et al. 2004).

Over the last decade, experimental evidence described the root apex structure as consisting of a transition zone (TZ) intercalated between two other regions—the apical division zone (DZ) and the elongation zone (EZ) (reviewed in Baluška et al. 2010). Cells traversing the TZ show a unique and specific cytoarchitecture, characterized by centralized post-mitotic nuclei surrounded by perinuclear microtubules radiating toward the cell periphery (Baluška et al. 1992, Baluška et al. 1996a). In contrast to the mitotically active DZ cells, which are continually assembling and disassembling mitotic spindles, the central cell bodies of the TZ are not engaged in such activities (Baluška et al. 2004a, Baluška et al. 2004b, Baluška et al. 2004c, Baluška et al. 2006) and thus perform other, more specific sensory activities (Mancuso et al. 2006, Wan et al. 2008). In fact, several pieces of experimental data suggest that the root apex TZ should be considered as a sort of sensory and information processing zone, enabling the growing root apex to monitor environmental parameters continuously and to trigger appropriate responses. The cells of the TZ are very sensitive to touch and extracellular calcium (Ishikawa and Evans 1992, Baluška et al. 1996), gravity and auxin (Mancuso et al. 2006, Sobol and Kordyum 2009), water and salt stress (Winch and Pritchard 1999, Wu and Cosgrove 2000, Ober and Sharp 2003), aluminum (Illéš et al. 2008, Amenòs et al. 2009, Marciano et al. 2010) and hypoxia (Mancuso and Boselli 2002, Mancuso and Marras 2006a, Mugnai et al. 2011).

Nitric oxide (NO) has emerged as a key signaling molecule in plants: it can be produced quickly and efficiently on demand, induces defined effects within the cells and is removed quickly when no longer required (Neill et al. 2003, Delledonne 2005, Lamotte et al. 2005). At the subcellular level, NO regulates the actin cytoskeleton, endocytosis, vesicle trafficking and the polarity of growing tip cells (Prado et al. 2004, Lombardo et al. 2006, Salmi et al. 2007, Prado et al. 2008, Kasprowicz et al. 2009, Wang et al. 2009). At the supracellular level, NO regulates root formation (Lamattina et al. 2003, Pagnussat et al. 2003, Pagnussat et al. 2004, Correa-Aragunde et al. 2004, Prado et al. 2004, Lanteri et al. 2008), seed germination (Beligni and Lamattina 2001), the induction of cell death (Pedroso et al. 2000), pathogen responses (Clarke et al. 2000, Chandok et al. 2003), stomatal regulation (Neill et al. 2003, Desikan et al. 2004, Neill et al. 2008) and photosynthesis (Takahashi and Yamasaki 2002). In addition, NO is widely implicated in the

plant response to environmental stress (Beligni and Lamattina 2001, Dat et al. 2004), but its exact role in the response of plants to hypoxia is still under evaluation (Dordas et al. 2003a, Stöhr and Stremlau 2006).

In the present work, we present experimental evidence for localized hypoxia sensing in the TZ of the maize root apex. Furthermore, we have investigated the role of localized NO emission from the TZ during the acclimation of the entire maize root to hypoxia applied locally to the TZ.

Results

The oxygen sensor is located in the root apex

Hypoxia induces a shift from aerobic respiration to fermentation; therefore, the activities of the main enzymes involved in the fermentative pathway from pyruvate to ethanol, namely alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC), were measured. The ADH and PDC activities indicated that the root apex plays the main role in inducing the adaptive response to hypoxia compared with the other root regions (**Fig. 1**). In fact, no significant variations in the activity of ADH and PDC were obtained when compartments A, B or C (**Fig. 2**) were independently subjected to oxygen deprivation vs. roots exposed to normoxic conditions. In contrast, measurements performed when compartment D (corresponding to the root apex) was subjected to hypoxia revealed a marked increase in the ADH and PDC activities in the entire root: the ADH activity increased 17-fold and the PDC activity increased

approximately 3-fold. These results strongly support the hypothesis that, when exposed to hypoxic conditions, only the root apex is capable of triggering a clear adaptive response, such as the increase in the fermentative activity in the entire root.

To discriminate between the roles of each root apex zone (DZ, TZ, and EZ) in the sensing process of the oxygen content in the surrounding environment, we have further measured the oxygen influxes at root level under both normoxic and hypoxic conditions. Under normoxic conditions, strong differences between the constituent zones were clearly evident. The TZ appeared to be the most active zone in the uptake of oxygen from the surrounding solution (**Fig. 3**). The spatial patterns of the oxygen influxes in the entire root apex showed a marked peak in the TZ ($110 \, pmol \, cm^{-2} \, s^{-1}$) at 1–2 mm from the maize root tip. A minor oxygen influx peak ($62 \, pmol \, cm^{-2} \, s^{-1}$) was also evident in the DZ. Importantly, with regards to oxygen influx, the TZ was the only root apex region significantly affected by oxygen deprivation; in fact, the marked peak of oxygen influx was greatly reduced under hypoxia, whereas the DZ maintained a similar pattern. Therefore, the TZ is the root apex zone that is the most sensitive to the sudden lack of oxygen that occurs during hypoxia and is a good candidate to be the main site for oxygen sensing in the root tip.

NO is implicated in the adaptive response of roots to hypoxic conditions

Hypoxic conditions also promoted NO production from the root apex. A burst of NO was suddenly produced after the onset of hypoxia (1–2 s), mostly from the TZ cells, reaching a
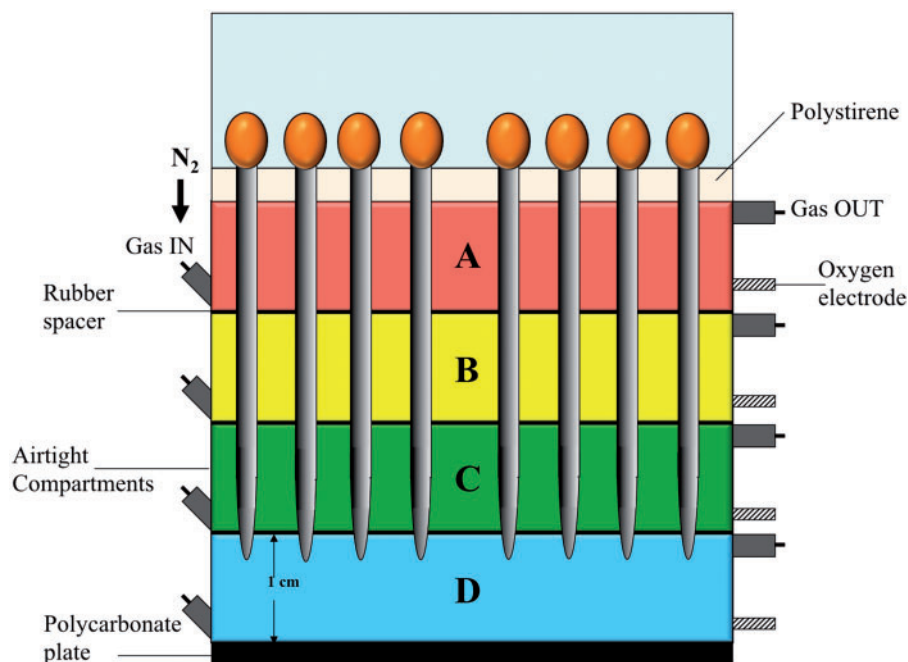

Fig. 1 Scheme of the growth chamber utilized to submit different regions of the root apex to hypoxic conditions. Each of the 4 different compartments of the growth chamber (A, B, C and D) can be separately made hypoxic by flowing it with N_2. Each compartment is 1 cm deep; each root apex penetrates in the D compartment for 4 mm.
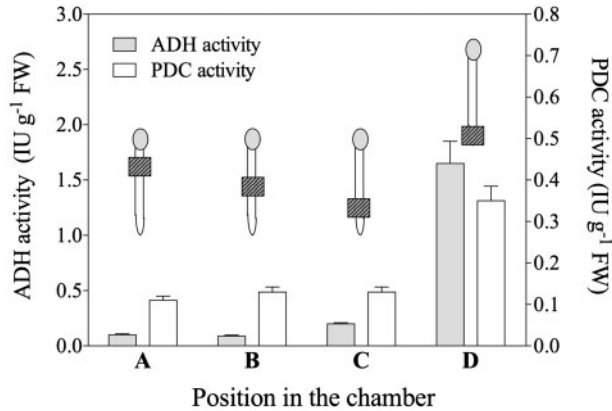
S. Mugnai *et al.*

Fig. 2 Acclimation requires local root apex hypoxia. ADH and PDC activities have been measured in the whole root after different regions of the roots were subjected to 1 h of hypoxia ([O$_2$] <10 nmol). ADH and PDC increase in the whole root just in the case that the root apex was subjected to hypoxia (D). Data are the means ± SD (*n* = 18). The position from A to D corresponds to the positions in the chamber represented in **Fig. 1**.

Fig. 3 Oxygen fluxes obtained by measurements in different regions of intact root apices (DZ, division zone; TZ, transition zone; EZ, elongation zone) under normoxic (filled circles) or hypoxic (open circles) conditions. Data are the means ± SD (*n* = 16).

peak of 1.1 pmol cm^{-2} s^{-1} after 1.5 min and lasting approximately 12 min before returning to the steady-state values (**Fig. 4**). Furthermore, the hypoxia-induced efflux of NO was strictly associated with the TZ. In fact, only a small and negligible efflux of NO (10-fold lower than that produced by the TZ) was detectable in the DZ, and, importantly, NO bursts were not detected in the EZ region. On a longer time scale, additional evidence of NO production in the root apex after the onset of hypoxia was visualized. The fluorescence produced by the NO-specific dye 4,5-diaminofluorescein diacetate (DAF-2DA) was detected after 30 and 60 min in the presence of anoxia or the NO scavenger 2(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) (**Fig. 5**)

NO donors and NO scavengers were used to investigate the possible involvement of NO in promoting an adaptive response to anoxic conditions during the hypoxic pre-treatment (HPT) of roots. A preliminary experiment was conducted to detect the most effective NO donor concentration to be used, e.g. the use of different soduim nitroprusside (SNP) concentrations (from 0 to 1,000 µM) confirmed that the effect of an NO donor was dose dependent. In the case of SNP, the maximal response was observed at 10 µM, with higher concentrations becoming toxic (**Fig. 6**). Some seedlings were previously subjected to an HPT (5 h of hypoxia at [O$_2$] = 30 nmol) before the anoxia treatment and then compared with seedlings not subjected to hypoxia pre-treatment (NHPT; **Fig. 7**) under anoxic conditions. As expected, the survival rates were completely different: almost all of the seedlings subjected to HPT survived, whereas only 48% of the seedlings subjected to NHPT remained alive. Exogenous NO was then supplied to seedlings subjected to NHPT at the root level using two different NO donors [SNP and *S*-nitroso, *N*-acetyl penicillamine (SNAP)]. Both of the NO donors increased the survival rate of the roots subjected to NHPT
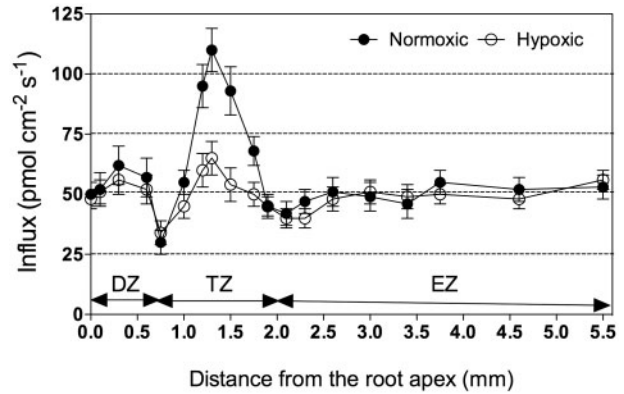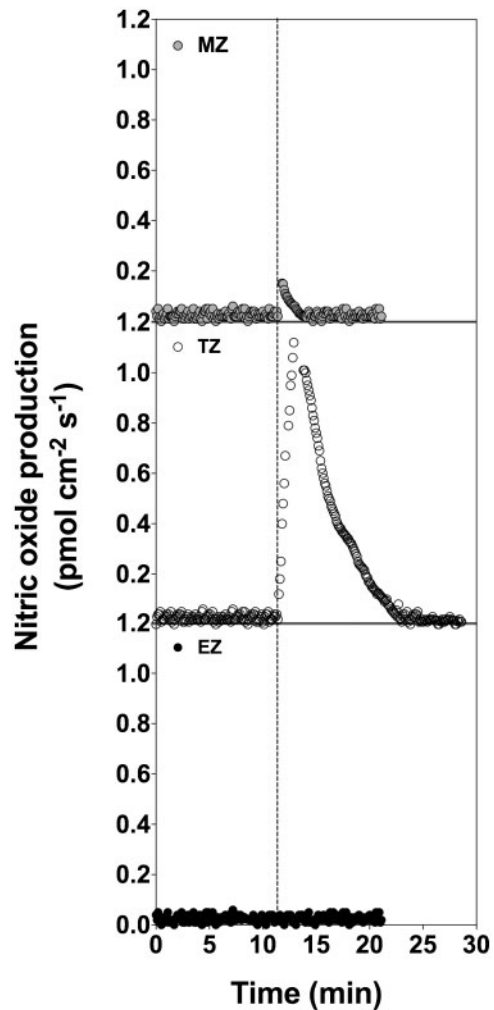

Fig. 4 A representative graph showing NO production in the different regions of the root apex after hypoxia (dotted line): DZ (Division Zone); TZ (Transition Zone); EZ Elongation Zone).
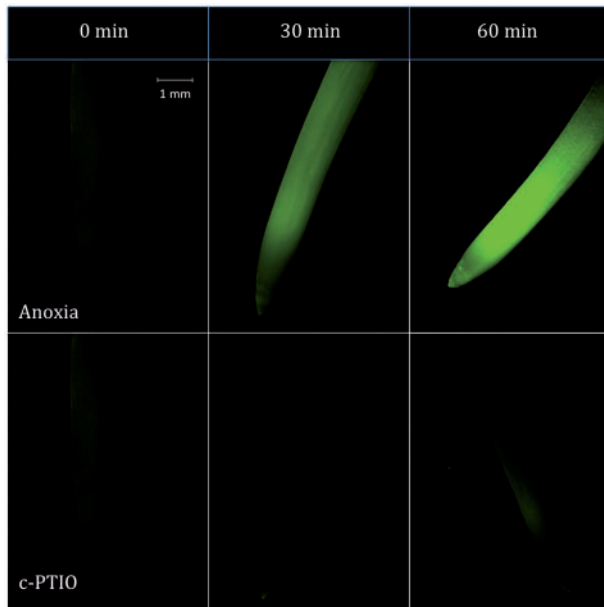
Fig. 5 NO production in mays roots exposed to different anoxia treatment (30 and 60 min). Three-day-old seedlings were loaded with 10 μM DAF-2A for 60 min and then subjected to anoxia for 30 and 60 min. Results are from one of four representative experiments.
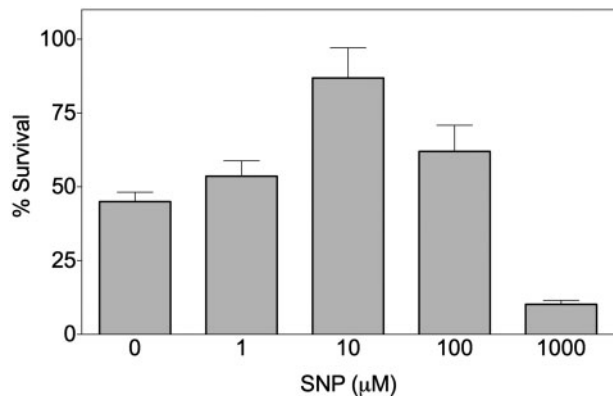

Fig. 6 Dose-dependent effect of SNP on the survival rate of maize roots under anoxia. Data are the means ± SD ($n = 23$).

Fig. 7 Effect of NO donors (SNP and SNAP) and a scavenger (cPTIO) on the survival rates of maize seedlings, previously subjected to hypoxic treatment (HPT) or not (NHPT), under anoxic conditions. Data are the means ± SD ($n = 18$).
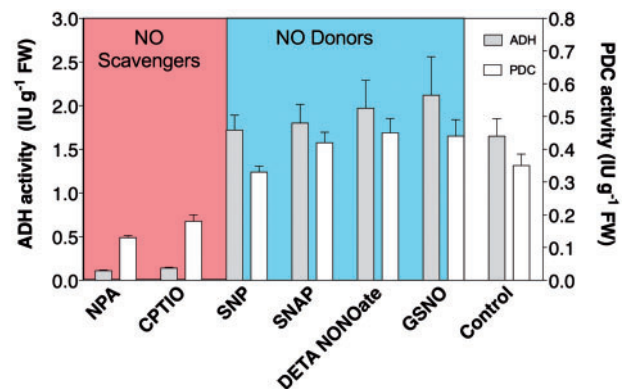
Fig. 8 ADH and PDC activities in the whole root after 1 h treatment of the root apex with NO donors in normoxic conditions and/or NO scavengers in hypoxic conditions ([O_2] <10 nmol). Control is under hypoxic conditions. Data are the means ± SD ($n = 22$).

undergoing anoxia at the level of the hypoxically treated roots (**Fig. 8**). Interestingly, the addition of a specific and powerful NO scavenger (cPTIO) to the HPT-exposed maize roots completely inhibited any effect of the HPT on the subsequent root survival under anoxic conditions. To test further the connection among the root apex, NO and hypoxic acclimation, we measured the activities of ADH and PDC in the entire root after the treatment of the root apex with different NO donors and scavengers. The results summarized in **Fig. 8** demonstrate that treating the root apex with several different NO donors induce a strong increase in the activities of ADH and PDC in the entire root, which was comparable with the increase obtained by hypoxic acclimation (**Fig. 2**). Conversely, the
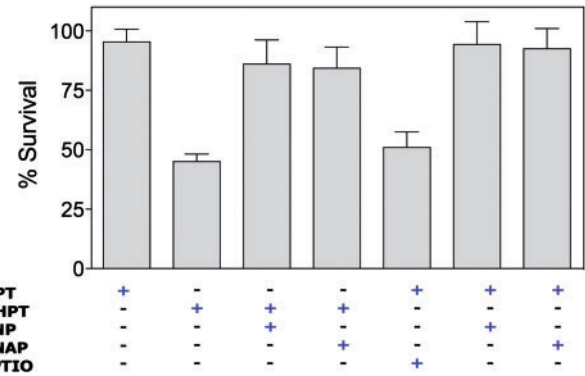
hypoxic treatment of the root apex in the presence of NO scavengers has no effect on the ADH and PDC activities in the root (**Fig. 8**).

Discussion

Roots are very efficient in mounting effective and adaptive responses when challenged with hypoxic or anoxic conditions in the soil (Drew 1997, Mancuso and Marras 2006a, Subbaiah 2009, Mugnai et al. 2011). Roots that sense a reduction of oxygen in the soil are able to detect areas with a better supply of oxygen and to grow towards these areas via so-called oxytropism, a term coined by Pfeffer in 1906 after the research of Molisch (1884) who first observed that roots submerged in water near the water–air interface reacted by growing upward towards the air. This oxytropic orientation of the root can also be induced by oxygen levels that are not low enough to stimulate hypoxic stress responses (Porterfield and Musgrave 1998),

suggesting that the elusive oxygen sensor of root apices is very sensitive.

The main site for oxygen sensing at the root level has been debated in the past (Dat et al. 2004, Bailey-Serres and Voesenek 2008). In the present study, we convincingly localized it to the TZ of the root apex, a region considered by some authors (Baluška et al. 2004a, Baluška et al. 2010) as a sort of 'command center' that processes sensory information from the surrounding environment and makes decisions about movements and adaptive root behavior. The TZ cells are involved in vigorous ion channel activities, endocytosis-driven vesicle trafficking and rapid cytoskeletal re-arrangements (Verbelen et al. 2006). The TZ exhibits the highest demand for oxygen in plant roots (Mancuso and Boselli 2002, McLamore et al. 2010a, this study). Although the exact nature of the processes requiring such a high amount of oxygen remains unclear, when the high activities of endocytosis and vesicle recycling are inhibited with either brefeldin A or aluminum (Illéš et al. 2006, Mancuso et al. 2006, Mancuso et al. 2007, Shen et al. 2008), the oxygen influx peak at this zone is also compromised. Similarly, the addition of exogenous auxin, which is known to inhibit endocytic vesicle recycling due to the inhibition of endocytosis (Paciorek et al. 2005), inhibits the transition zone oxygen influx peak and root growth (McLamore et al. 2010b).

Because all of the processes occurring in the TZ consume a large amount of ATP, the finding that the TZ cells require the highest amount of oxygen in the entire root apex, much more than the neighboring regions, is reasonable (**Fig. 3**). Accordingly, the TZ appears to be the most sensitive part of the root apex under hypoxic conditions (**Fig. 3**). In fact, when selectively subjected to hypoxia, the root apex is able to elicit an acclimation response in the entire root (**Fig. 2**).

The production of NO is involved in stress-induced adaptation (An et al. 2005, Wang and Yang 2005, Sun et al. 2007), and the present data reveal that the oxygen deprivation of roots induces local NO emission, particularly in the TZ. In addition, this local NO production peak is essential for the successful acclimation of the entire maize root to oxygen deprivation.

Oxygen sensing in plants via the evolutionarily conserved N-end rule pathway has recently been discovered (Gibbs et al. 2011, Licausi et al. 2011). The ethylene response factor (ERF)-transcription factor RAP2.12 was shown to be released from the plasma membrane under hypoxia and to accumulate in the nucleus to activate gene expression for the acclimation to hypoxia (Licausi et al. 2011). The N-end rule pathway of targeted proteolysis acts as a homeostatic sensor of hypoxia in Arabidopsis (Sasidharan and Mustroph 2011), and NO and the hypoxia-inducible oxygen-binding plant hemoglobins have been suggested to be involved in oxygen sensing (Sowa et al. 1998, Dordas et al. 2003b, Igamberdiev and Hill 2004, Borisjuk and Rolletschek 2008, Thiel et al. 2011). Moreover, PAS domain proteins are known to be regulated either by oxygen or by redox changes (Gilles-Gonzalez and Gonzalez 2004); there are several PAS domain proteins present in plants, but they appear to be mostly involved in light sensing.

In animals, NO and NO-derived molecules exert their biological actions through the specific post-translational chemical modification of protein targets (Besson-Bard et al. 2008). To date, >100 proteins have been identified as targets for NO in vitro and/or in vivo in plant tissues. The large panel of functions assigned to NO target proteins essentially concerns all of the main cellular activities, especially signaling. In *Vitis*, the ability of HPT to modify protein synthesis is a well-known phenomenon (Mancuso and Marras 2006a, Shabala et al. 2006, Mugnai et al. 2011), with a primary role for NO in the acclimation process, as confirmed in our study by the use of NO donors and scavengers (**Fig. 7**).

The tolerance to anoxia is usually much better expressed when the tissues are first acclimated during HPT rather that after a sudden transfer to anoxia. A number of polypeptides expressed under HPT have been identified as enzymes (Sachs et al. 1980) that trigger the metabolic shift from oxidative phosphorylation to anerobic fermentation. However, the nature of the signal for the induction of this metabolic adaptation to low O_2 conditions is still under debate. From our results, NO appears to be one of the primary signals emitted by plants during hypoxia, confirming the hypothesis of Dordas et al. (2003b).

The exposure of growing roots to microgravity is known to induce the root apex hypoxia response (Paul et al. 2001, Stout et al. 2001), and the subcellular localization of microgravity-induced ADH revealed the cross-walls at the root apex TZ as the subcellular domains that suffer the most when exposed to oxygen deficiency under microgravity conditions (Paul et al. 2001, Stout et al. 2001). Importantly, other plant organs and tissues do not show signs of oxygen deficiency under microgravity (Paul et al. 2001), a situation that is different from that on Earth (under normal gravity) in which the hypoxia experienced solely by the roots also induces hypoxia stress responses in the shoots (Chung and Ferl 1999). Until now, it has not been easy to understand this local root apex hypoxia, but our data could provide the missing clue. In fact, the very high demand for oxygen in the TZ is not easily fulfilled under microgravity conditions due to problems with the diffusion of gases in solution under conditions of microgravity (Porterfield 2002, Liu et al. 2004).

In conclusion, the putative oxygen sensor induces the rapid local emission of NO in the TZ when maize roots are exposed to hypoxia. This hypoxia-induced NO emission functions to protect the root apex cells and also to lower the oxygen demand in the TZ. Our previously published data show that NO depolymerizes F-actin (Kasprowicz et al. 2009) and inhibits endocytosis and endocytic vesicle recycling (Kasprowicz et al. 2009 for *Zea mays* roots; Wang et al. 2009 for *Pinus bungeana* pollen tubes). Our unpublished data identify endocytic vesicle recycling as the major oxygen-demanding process in the root apex. Therefore, we
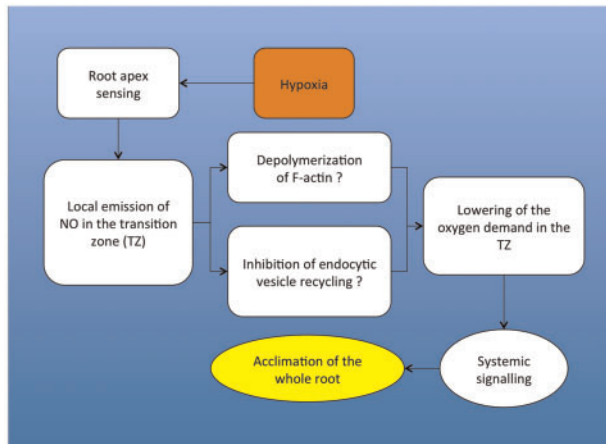
Fig. 9 A generalized model of the auto-regulatory loop between NO emission and oxygen consumption in the transition zone of the root apex.

propose that the hypoxia-induced NO is lowering the oxygen demand peak in the transition zone via the inhibition of actin polymerization and endocytic vesicle recycling. This lowered oxygen demand then induces, through an as yet unknown process, systemic signaling throughout the root, allowing the hypoxic acclimation of the entire root (**Fig. 9**). Therefore, this NO emission–oxygen consumption autoregulatory loop of the TZ can effectively adapt the root physiology to the local oxygen availability. NO acts as a protector for hypoxia-stressed maize roots, as similarly reported for ABA and low water potential (Ober and Sharp 2003). Future studies will be aimed at the further characterization of this unique root apex zone, which translates sensory information into motor behavior.

Materials and Methods

Plant material and treatments

Caryopses of Z. *mays* L. cv. Gritz (Maïsadour Semences) were soaked overnight in aerated tap water and placed between damp paper towels in Petri dishes; the dishes were maintained in a vertical position and incubated at 24°C for 48 h. When the seedling roots reached a length of approximately 4 cm (usually after 3 d), they were placed in a makeshift chamber for the hypoxia/anoxia treatments. **Fig. 1** describes the method used to separate the different parts of the seminal root hermetically to evaluate the effect of hypoxia/anoxia treatments. At specific times, each sealed compartment (A, B, C and D) was subjected to hypoxia by introducing nitrogen until an oxygen concentration inside the compartment of <30 nmol was reached, whereas the other three compartments maintained ambient levels of oxygen. Compartment D referred to the root apex (up to 5 mm from the root tip), whereas compartments A, B and C comprised the mature zones of the root. An oxygen

electrode (OX-50, Unisense) measured the actual content of oxygen in each compartment.

ADH and PDC activities

The activities of ADH and PDC were assessed after 5 h of hypoxia at approximately 30 nmol of oxygen. Samples of 200 mg of root tissue were ground into a fine powder in liquid nitrogen and extracted using 50 mM HEPES-KOH (pH 6.8) containing 5 mM Mg acetate, 5 mM β-mercaptoethanol, 15% (v/v) glycerine, 1 mM EDTA, 1 mM EGTA, 5 mM dithiothreitol (DTT) and 0.1 mM Pefabloc proteinase inhibitor (Boehringer Mannheim), as described by Biemelt et al. (1999). The homogenate was centrifuged at $13,000 \times g$ at 4°C for 15 min, and the resulting supernatant was used for the spectrophotometric determination of the activities of the different enzymes at 340 nm. The activities of ADH and PDC were measured as described by Mustroph and Albrecht (2003).

Survival determination

Maize plants with primary roots of uniform lengths (3–5 cm) were arranged in a plastic holder at the top of a Plexiglas box containing nutrient solution. Following the method adopted by Verslues et al. (1998), the roots were left to grow downward through transparent root guides made from plastic drinking straws (i.d. of 7 mm). The root guides were perforated with several holes (diameter of 1 mm) to allow the exchange of solution. The solution was vigorously aerated with air. Hypoxia was achieved by introducing nitrogen until an oxygen concentration inside the compartment of <30 nmol was reached. The roots were considered to have survived if they resumed elongation after the anoxic treatment. Under aerated conditions, the roots showed no symptoms of damage.

Oxygen fluxes

Oxygen flux measurements were performed under normoxic and hypoxic conditions ($[O_2]$ <10 nmol) using the vibrating probe technique (Baluška et al. 2008, Schlicht et al. 2008). Briefly, healthy Z. *mays* L. root apices (5–6 mm long) were cut, carefully washed with deionized water and placed individually at the bottom of a measuring chamber containing an electrophysiological solution (10 mM $CaCl_2$, pH 6.5). The flux measurement was performed at 24 ± 0.25°C by positioning a custom-built oxygen-selective microelectrode (tip diameter of 1 µm; Mancuso et al. 2000, Mancuso and Marras 2003) near the root surface. During the recording, the microelectrode oscillated in a square wave parallel to the electrode axis over a distance of 10 µm (0.1 Hz frequency), moving along the entire root length. The calculation of the difference between the voltage of each electrode position and that of the previous one at the other extreme position as well as the evaluation of a moving average of these differences over any desired time period, producing the potential difference, were computer generated. The O_2 influxes were calculated using Fick's first law of diffusion, assuming a

NO production

To localize the production of NO in the different regions of the root apex with a spatial resolution of a few micrometers, an NO-selective microelectrode of carbon fibers with diameters as small as 5 μm was constructed (Mancuso and Marras 2006b). The dimensions and the response time (<0.5 s) allowed the use of this electrode in a self-referencing mode (Mancuso et al. 2000, Mancuso and Boselli 2002), with a resolution as small as 50 fmol cm^{-2} s^{-1}.

The NO content in the root apices was also determined microscopically. Briefly, caryopses of *Z. mays* cv. Kubrick were placed between damp paper towels in Petri dishes maintained in a vertical position and incubated at 26°C for 3 d. The seedlings were loaded in 10 μM DAF-2DA (Sigma-Aldrich) in HEPES-NaOH (pH 7.5) for 60 min at 24°C in the dark. After 60 min, the seedlings were washed with HEPES-NaOH buffer to remove the excessive fluorophore. The seedlings where then exposed to anoxia for 30 or 60 min at 24°C. The DAF-2DA-dependent fluorescence was detected using a fluorescent stereomicroscope (Zeiss), and the digital images were captured using a cooled CCD camera controlled by Axiovision software (Zeiss).

Chemicals

SNP, SNAP, DETA NONOate (diethylenetriamine NONOate) and GSNO (*S*-nitrosoglutathione) were used as NO donors; cPTIO was used as an NO scavenger and NPA (*N*-propyl-L-arginine) as a nitric oxide synthase inhibitor. All of the chemicals were obtained from Sigma-Aldrich. The amount of NO released from the donor solutions was determined using the Griess reagent colorimetric kit (Cayman Chemical Company) according to the manufacturer's instructions and was within the range described in the literature from nanomolar to low micromolar amounts (Ferrer and Ros Barcelò, 1999).

References

Aiken, R.M. and Smucker, A.J.M. (1996) Root system regulation of whole plant growth. *Annu. Rev. Phytopathol.* 34: 325–346.

Amenòs, M., Corrales, I., Poschenrieder, C., Illès, P., Baluška, F. and Barcelò, J. (2009) Different effects of aluminum on the actin cytoskeleton and brefeldin A-sensitive vesicle recycling in root apex cells of two maize varieties differing in root elongation rate and aluminum tolerance. *Plant Cell Physiol.* 50: 528–540.

An, L., Liu, Y., Zhang, M., Chen, T. and Wang, X. (2005) Effects of nitric oxide on growth of maize seedling leaves in the presence or absence of ultraviolet-B radiation. *J. Plant Physiol.* 162: 317–326.

Bailey-Serres, S. and Voesenek, L.A.C.J. (2008) Flooding stress: acclimations and genetic diversity. *Annu. Rev. Plant Biol.* 59: 313–339.

Baluška, F., Barlow, P.W., Parker, J.S. and Volkmann, D. (1996) Symmetric reorganizations of radiating microtubules around pre-mitotic and post-mitotic nuclei of dividing cells organized within intact root meristems. *J. Plant Physiol.* 149: 119–128.

Baluška, F., Mancuso, S., Volkmann, D. and Barlow, P.W. (2004a) Root apices as plant command centres: the unique 'brain-like' status of the root apex transition zone. *Biologia* 59: 7–19.

Baluška, F., Mancuso, S., Volkmann, D. and Barlow, P.W. (2010) Root apex transition zone: a signalling–response nexus in the root. *Trends Plant Sci.* 15: 402–408.

Baluška, F., Parker, J.S. and Barlow, P.W. (1992) Specific patterns of cortical and endoplasmic microtubules associated with cell growth and tissue differentiation in roots of maize (*Zea mays* L.). *J. Cell Sci.* 103: 191–200.

Baluška, F., Schlicht, M., Volkmann, D. and Mancuso, S. (2008) Vesicular secretion of auxin: evidences and implications. *Plant Signal. Behav.* 3: 254–256.

Baluška, F., Volkmann, D. and Barlow, P.W. (2004b) Cell bodies in a cage. *Nature* 428: 371.

Baluška, F., Volkmann, D. and Barlow, P.W. (2004c) Eukaryotic cells and their cell bodies: cell theory revisited. *Ann. Bot.* 94: 9–32.

Baluška, F., Volkmann, D. and Barlow, P.W. (2006) Cell–cell channels and their implications for cell theory. *In* Cell–Cell Channels. Edited by Baluška, F., Volkmann, D. and Barlow, P.W. pp. 1–18. Landes Bioscience and Springer Verlag.

Beligni, M.V. and Lamattina, L. (2001) Nitric oxide in plants: the history is just beginning. *Plant Cell Environ.* 24: 267–278.

Besson-Bard, A., Pugin, A. and Wendehenne, D. (2008) New insights into nitric oxide signalling in plants. *Annu. Rev. Plant Biol.* 59: 21–39.

Biemelt, S., Hajirezaei, M.R., Melzer, M., Albrecht, G. and Sonnewald, U. (1999) Sucrose synthase activity does not restrict glycolysis in roots of transgenic potato plants under hypoxic conditions. *Planta* 210: 41–49.

Borisjuk, L. and Rolletschek, H. (2008) Nitric oxide is a versatile sensor of low oxygen stress in plants. *Plant Signal. Behav.* 3: 391–193.

Chandok, M.R., Ytterberg, A.J., van Wijk, K.J. and Klessig, D.F. (2003) The pathogen-inducible nitric oxide synthase (iNOS) in plants is a variant of the p protein of the glycine decarboxylase complex. *Cell* 13: 469–482.

Chung, H.-J. and Ferl, R.J. (1999) Arabidopsis alcohol dehydrogenase expression in both shoots and roots is conditioned by root growth environment. *Plant Physiol.* 121: 429–436.

Clarke, A., Desikan, R., Hurst, R.D., Hancock, J.T. and Neill, S.J. (2000) NO way back: nitric oxide and programmed cell death in Arabidopsis thaliana suspension cultures. *Plant J.* 24: 667–677.

Correa-Aragunde, N., Graziano, M. and Lamattina, L. (2004) Nitric oxide plays a central role in determining lateral root development in tomato. *Planta* 218: 900–905.

Dat, J.F., Capelli, N., Folzer, H., Bourgeade, P. and Badot, P.M. (2004) Sensing and signalling during plant flooding. *Plant Physiol. Biochem.* 42: 273–282.

Delledonne, M. (2005) NO news is good news for plants. *Curr. Opin. Plant Biol.* 8: 390–396.

cylindrical diffusion geometry. The flux measurements were performed on at least 10 different root apices per treatment ($n \geq 10$).

Desikan, R., Cheung, M.K., Bright, J., Henson, D., Hancock, J.T. and Neill, S.J. (2004) ABA, hydrogen peroxide, and nitric oxide signalling in stomatal guard cells. *J. Exp. Bot.* 55: 205–212.

Dordas, C., Hasinoff, B., Igamberdiev, A.U., Manac'h, N., Rivoal, J. and Hill, R.D. (2003a) Expression of a stress-induced hemoglobin affects NO levels produced by alfalfa root cultures under hypoxic stress. *Plant J.* 35: 763–770.

Dordas, C., Rivoal, J. and Hill, R.D. (2003b) Plant haemoglobins, nitric oxide and hypoxic stress. *Ann. Bot.* 91: 173–178.

Drew, M.C. (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 223–250.

Ferrer, M.A. and Ros Barcelò, A.R. (1999) Differential effects of nitric oxide on peroxidase and H_2O_2 production by the xylem of *Zinnia elegans*. *Plant Cell Environ.* 22: 891–897.

Gibbs, D.J., Lee, C.S., Isa, NM., Gramuglia, S., Fukao, T., Bassel, G.W. et al. (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* 479: 415–418.

Gilles-Gonzalez, M.A. and Gonzalez, G. (2004) Signal transduction by heme-containing PAS-domain proteins. *J. Appl. Physiol* 96: 774–783.

Igamberdiev, A.U. and Hill, R.D. (2004) Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. *J. Exp. Bot.* 55: 2473–2482.

Illéš, P., Schlicht, M., Pavlovkin, J., Lichtscheidl, I., Baluška, F. and Ovečka, M. (2006) Aluminium toxicity in plants: internalisation of aluminium into cells of the transition zone in Arabidopsis root apices relates to changes in plasma membrane potential, endosomal behaviour, and nitric oxide production. *J. Exp. Bot.* 57: 4201–4213.

Ishikawa, H. and Evans, M.L. (1992) Induction of curvature in maize roots by calcium or by thigmostimulation. Role of postmitotic isodiametric growth zone. *Plant Physiol.* 100: 762–768.

Kasprowicz, A., Szuba, A., Volkmann, D., Baluška, F. and Wojtaszek, F. (2009) Nitric oxide modulates dynamic actin cytoskeleton and vesicle trafficking in a cell type-specific manner in root apices. *J. Exp. Bot.* 60: 1605–1617.

Lamattina, L., García-Mata, C., Graziano, M. and Pagnussat, G. (2003) Nitric oxide: the versatility of an extensive signal molecule. *Annu. Rev. Plant. Biol.* 54: 109–136.

Lamotte, O., Courtois, C., Barnavon, L., Pugin, A. and Wendehenne, D. (2005) Nitric oxide in plants: the biosynthesis and cell signalling properties of a fascinating molecule. *Planta* 221: 1–4.

Lanteri, M.L., Laxalt, A.M. and Lamattina, L. (2008) Nitric oxide triggers phosphatidic acid accumulation via phospholipase D during auxin-induced adventitious root formation in cucumber. *Plant Physiol.* 147: 188–198.

Licausi, F., Kosmacz, M., Weits, D.A., Giuntoli, B., Giorgi, F.M., Voesenek, L.A.C.J. et al. (2011) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* 479: 419–422.

Liu, L.J., Monje, O., Stutte, G.W. and Porterfield, D.M. (2004) Induction of hypoxic root metabolism results from physical limitations in O_2 bioavailability in microgravity. *Adv. Space Res.* 34: 1579–1584.

Lombardo, M.C., Graziano, M., Polacco, J.C. and Lamattina, L. (2006) Nitric oxide functions as a positive regulator of root hair development. *Plant Signal. Behav.* 1: 28–33.

Mancuso, S., Barlow, P.W., Volkmann, D. and Baluška, F. (2006) Actin turnover-mediated gravity response in maize root apices:

gravitropism of decapped roots implicates gravisensing outside of the root cap. *Plant Signal. Behav.* 1: 52–58.

Mancuso, S. and Boselli, M. (2002) Characterisation of the oxygen fluxes in the division, elongation and mature zones of Vitis roots: influence of oxygen availability. *Planta* 214: 767–774.

Mancuso, S. and Marras, A.M. (2003) Different pathways of the oxygen supply in the sapwood of young Olea europaea trees. *Planta* 216: 1028–1033.

Mancuso, S. and Marras, A.M. (2006a) Adaptative response of Vitis root to anoxia. *Plant Cell Physiol.* 47: 401–409.

Mancuso, S. and Marras, A.M. (2006b) New solid state microsensors in plant physiology. *In* Plant Electrophysiology. Edited by Volkov, A. pp. 155–172. Springer, Berlin.

Mancuso, S., Marras, A.M., Mugnai, S., Schlicht, M., Žársky, V., Li, G. et al. (2007) Phospholipase Dζ2 drives vesicular secretion of auxin for its polar cell–cell transport in the transition zone of the root apex. *Plant Signal. Behav.* 2: 240–244.

Mancuso, S., Marras, A.M. and Papeschi, G. (2000) A polarographic, oxygen-selective, vibrating-microelectrode system for the spatial and temporal characterisation of transmembrane oxygen fluxes in plants. *Planta* 211: 384–389.

Marciano, D.P.R.O., Toledo-Ramos, F., Neiva-Alvim, M., Magalhaes, J.R. and Costa França, M.G. (2010) Nitric oxide reduces the stress effects of aluminum on the process of germination and early root growth of rice. *J. Plant Nutr. Soil Sci.* 173: 885–891.

McLamore, E.S., Diggs, A., Calvo Marzal, P., Shi, J., Blakeslee, J.J., Peer, W.A. et al. (2010b) Non-invasive quantification of endogenous root auxin transport using an integrated flux microsensor technique. *Plant J.* 63: 1004–1016.

McLamore, E.S., Jaroch, D., Chatni, M.R. and Porterfield, D.M. (2010a) Self-referencing optrodes for measuring spatially resolved, real-time metabolic oxygen flux in plant systems. *Planta* 232: 1087–1099.

Molisch, H. (1884) Ueber die Ablenkung der Wurzeln von ihrer normalen Wachsthumsrichtung durch Gase (Aerotropismus). *Ber. Dtsch. Bot. Ges.* 2: 160–169.

Mugnai, S., Marras, A.M. and Mancuso, S. (2011) Effect of hypoxic acclimation on anoxia tolerance in *Vitis* roots: response of metabolic activity and K^+ fluxes. *Plant Cell Physiol.* 52: 1107–1116.

Mustroph, A. and Albrecht, G. (2003) Tolerance of crop plants to oxygen deficiency stress: fermentative activity and photosynthetic capacity of entire seedlings under hypoxia and anoxia. *Physiol. Plant.* 117: 508–520.

Neill, S., Barros, R., Bright, J., Desikan, R., Hancock, J., Harrison, J. et al. (2008) Nitric oxide, stomatal closure, and abiotic stress. *J. Exp. Bot.* 59: 165–176.

Neill, S.J., Desikan, R. and Hancock, J.T. (2003) Nitric oxide signalling in plants. *New Phytol.* 159: 11–35.

Ober, E.S. and Sharp, R.E. (2003) Electrophysiological responses of maize roots to low water potentials: relationship to growth and ABA accumulation. *J. Exp. Bot.* 54: 813–824.

Paciorek, T., Zazímalová, E., Ruthardt, N., Petrásek, J., Stierhof, Y.D., Kleine-Vehn, J. et al. (2005) Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* 435: 1251–1256.

Pagnussat, G.C., Lanteri, M.L. and Lamattina, L. (2003) Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiol.* 132: 1241–1248.

Pagnussat, G.C., Lanteri, M.L., Lombardo, M.C. and Lamattina, L. (2004) Nitric oxide mediates the indole acetic acid induction activation of a mitogen-activated protein kinase cascade

involved in adventitious root development. *Plant Physiol.* 135: 279–286.

Paul, A.L., Daugherty, L., Bihn, E.A., Chapman, D.K., Norwood, K.L.L. and Ferl, R.J. (2001) Transgene expression patterns indicate that spaceflight affects stress signal perception and transduction in Arabidopsis. *Plant Physiol.* 126: 613–621.

Pedroso, M.C., Magalhaes, J.R. and Durzan, D. (2000) Nitric oxide induces cell death in *Taxus* cells. *Plant Sci.* 157: 173–180.

Pfeffer, W. (1906) Tropic movements. *In* The Physiology of Plants: A Treatise Upon the Metabolism and Sources of Energy in Plants, Vol. 3. Edited by Ewart, A.J. p. 180. The Clarendon Press, Oxford.

Porterfield, D.M. (2002) The biophysical limitations in physiological transport and exchange in plants grown in microgravity. *J. Plant Growth Regul.* 21: 177–190.

Porterfield, D.M. and Musgrave, M.E. (1998) The tropic response of plant roots to oxygen: oxytropism in *Pisum sativum* L. *Planta* 206: 1–6.

Prado, A.M., Colaço, R., Moreno, N., Silva, A.C. and Feijó, J.A. (2008) Targeting of pollen tubes to ovules is dependent on nitric oxide (NO) signaling. *Mol. Plant* 1: 703–714.

Prado, A.M., Porterfield, D.M. and Feijó, J.A. (2004) Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. *Development* 131: 2707–2714.

Sachs, M.M., Freeling, M. and Okimoto, R. (1980) The anaerobic proteins of maize. *Cell* 20: 761–767.

Salmi, M.L., Morris, K.E., Roux, S.J. and Porterfield, D.M. (2007) Nitric oxide and cGMP signaling in calcium-dependent development of cell polarity in *Ceratopteris richardii*. *Plant Physiol.* 144: 94–104.

Sasidharan, R. and Mustroph, A. (2011) Plant oxygen sensing is mediated by the N-end rule pathway: a milestone in plant anaerobiosis. *Plant Cell* 23: 4173–4183.

Schlicht, M., Šamajová, O., Schachtschabel, D., Mancuso, S., Menzel, D., Boland, W. et al. (2008) D'orenone blocks polarized tip-growth of root hairs by interfering with the PIN2-mediated auxin transport network in the root apex. *Plant J.* 55: 709–717.

Shabala, S., Shabala, L., Gradmann, D., Chen, Z., Newman, I. and Mancuso, S. (2006) Oscillations in plant membrane-transport activity: model predictions, experimental validation, and physiological implications. *J. Exp. Bot.* 57: 171–184.

Shen, H., Hou, N.Y., Schlicht, M., Wan, Y., Mancuso, S. and Baluška, F. (2008) Aluminium toxicity targets PIN2 in Arabidopsis root apices: effects on PIN2 endocytosis, vesicular recycling, and polar auxin transport. *Chin. Sci. Bull.* 53: 2480–2487.

Sobol, M. and Kordyum, E. (2009) Distribution of calcium ions in cells of the root distal elongation zone under clinorotation. *Microgravity Sci. Technol.* 21: 179–185.

Sowa, A.W., Stephan, M., Duff, G., Guy, P.A. and Hill, R.D. (1998) Altering hemoglobin levels changes energy status in maize cells under hypoxia. *Proc. Natl Acad. Sci. USA* 95: 10317–10321.

Stöhr, C. and Stremlau, S. (2006) Formation and possible roles of nitric oxide in plant roots. *J. Exp. Bot.* 57: 463–470.

Stout, S.C., Porterfield, D.M., Briarty, L.G., Kuang, A. and Musgrave, M.E. (2001) Evidence of root zone hypoxia in *Brassica rapa* L. grown in microgravity. *Int. J. Plant Sci.* 162: 249–255.

Subbaiah, C.C. (2009) Ionic loops and rebounds: oxygen-deprivation signaling in plants. *In* Signaling in Plants. Edited by Mancuso, S. and Baluška, F. pp. 195–207. Springer, Berlin.

Sun, B.T., Jing, Y., Chen, K.M., Song, L.L., Chen, F.J. and Zhang, L.X. (2007) Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*). *J. Plant Physiol.* 164: 536–543.

Takahashi, S. and Yamasaki, H. (2002) Reversible inhibition of photophosphorylation in chloroplasts by nitric oxide. *FEBS Lett.* 512: 145–148.

Thiel, J., Rolletschek, H., Friedel, S., Lunn, J.E., Nguyen, T.H., Feil, R. et al. (2011) Seed-specific elevation of non-symbiotic hemoglobin AtHb1: beneficial effects and underlying molecular networks in Arabidopsis thaliana. *BMC Plant Biol.* 11: 48.

Verbelen, J.P., De Cnodder, T., Le, J., Vissenberg, K. and Baluška, F. (2006) The root apex of *Arabidopsis thaliana* consists of four distinct zones of cellular activities: meristematic zone, transition zone, fast elongation zone, and growth terminating zone. *Plant Signal. Behav.* 1: 296–304.

Verslues, P.E., Ober, E.S. and Sharp, R.E. (1998) Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiol.* 116: 1403–1412.

Wan, Y., Eisinger, W., Ehrhardt, D., Baluška, F. and Briggs, W. (2008) The subcellular localization and blue-light-induced movement of phototropin 1–GFP in etiolated seedlings of *Arabidopsis thaliana*. *Mol. Plant* 1: 103–117.

Wang, Y., Chen, T., Zhang, C., Hao, H., Liu, P., Zheng, M. et al. (2009) Nitric oxide modulates the influx of extracellular Ca^{2+} and actin filament organization during cell wall construction in *Pinus bungeana* pollen tubes. *New Phytol.* 182: 851–862.

Wang, Y.S. and Yang, Z.M. (2005) Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of *Cassia tora* L. *Plant Cell Physiol.* 46: 1915–1923.

Winch, S. and Pritchard, J. (1999) Acid-induced cell wall loosening is confined to the accelerating region of the root growing zone. *J. Exp. Bot.* 50: 1481–1487.

Wu, Y. and Cosgrove, D.J. (2000) Adaptations of roots to low water potentials by changes in cell wall extensibility and cell wall properties. *J. Exp. Bot.* 50: 1543–1553.